IDENTIFICATION OF GENETIC FACTORS RESPONSIBLE FOR RARE DISORDERS WITH CONGENITAL HEART DEFECTS

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Congenital Heart Defects (CHDs) are individually rare, but altogether affect 6-8/1000 newborns. Their aetiologies are largely unknown, although mutations in a number of genes have been identified in few sporadic and familial cases. A total of 238 patients affected by different conotruncal (CT) CHDs were collected. These patients were screened for mutations in cardiogenic transcription factors NKX2.5, GATA4, ISLET1, TBX20 and ZFPM2/FOG2, together with other candidate genes expressed in developing and adult heart (GJA5, GDF1 and BMP4). A subgroup of 49 patients with tetralogy of Fallot (ToF) was further analyzed for JAG1 mutations, the gene responsible for Alagille syndrome. Mutation screening was performed by dHPLC followed by bidirectional sequencing. The GATA4 gene was further investigated for copy-number-changes by MLPA. No clear pathogenic mutation was identified in the GATA4, ISLET1, BMP4, TBX20 and GDF1 genes. One NKX2.5 (Arg25Cys) change was detected in 2 patients with ToF. Mutation analysis of ZFPM2/FOG2 gene identified 3 novel (Ile227Val, Met544Ile and Lys1029Ile) and one (Glu30Gly) previously detected mutations. One GJA5 missense mutation was identified in two unrelated patients. This change was absent in more than 400 control chromosomes and was proven to alter connexin 40 cellular localization by immunohistochemistry. One splice site variant (1107+3InsGT) and 2 missense mutations (G309R and R937Q) were found in JAG1 gene in 3 ToF patients. These results i) confirm that mutations in NKX2.5, GATA4, FOG2, TBX20, and GDF1 are rare events in CT CHDs ii) indicate a role for GJA5 gene in CT CHDs; iii) provide evidence that mutations in JAG1 gene account for a significant proportion of isolated ToF.

Probands of 7 families with isolated transposition of great arteries (TGA) and family history of concordant or discordant CHDs and 20 sporadic patients with atrioventricular canal defect (AVCD: 17 with partial and 3 with complete AVCD) were screened for mutations in the ZIC3, ACVR2B, LEFTYA, CFC1, NODAL, FOXH1, GDF1, CRELD1, GATA4 and NKX2.5 genes. Mutation analysis allowed the identification of 5 sequence variations in 2 (28.6%) out of 7 TGA probands, IVS2-1G>C (NODAL), Asn21His and Arg47Gln (CFC1), Pro21Ser (FOXH1), and Gly17Cys (ZIC3). The Asn21His and Arg47G CFC1 changes were also detected in 2/3 (66.7%) cases with complete AVCD. No variation was found in patients with partial AVCD. None of these changes was found in control subjects (n. 300), excepting for CFC1 variations Asn21His and Arg47Gln (2/300; 0.7%). These results demonstrate that mutations in laterality genes could occur in a significant proportion of families with TGA and argue for an oligogenic or complex mode of inheritance in these pedigrees.

To evaluate whether the GJA5 gene mutations cause "criss-cross" heart development in humans, we screened the entire coding region of the GJA5 gene in a group of 6 well characterized patients with criss-cross heart. No pathogenic mutation was identified, suggesting that GJA5 mutations are not responsible for criss-cross heart in humans or are not a major cause for this defect. To evaluate the presence of a locus for non syndromic absent pulmonary valve (APV) on 18q and for Ebstein anomaly on chromosome 8p23, we screened for mutations the NFATC1 and GATA4 genes in 2 subjects affected by isolated non syndromic APV and 7 patients with Ebstein anomaly, respectively. Neither NFATC1 nor GATA4 mutations were identified in these CHDs. Furthermore, a total of 30 patients affected by AVCD and a group of 50 phenotypically normal subjects were analyzed for NFATC1 mutations. Two non-synonymous changes (Val210Met and Ala367Val) were detected in 3 AVCD patients, while no mutation was detected in unaffected subjects. Both mutations were found to be absent in additional 400 controls, suggesting that NFATC1 gene could be responsible for a number of AVCD patients.

To further evaluate the mutation spectrum associated to Noonan Syndrome (NS), Costello Syndrome (CS), Cardio-Facio-Cutaneous Syndrome (CFCS) and LEOPARD Syndrome (LS) causative genes encoding for members of the RAS-MAPK pathway were studied by dHPLC analysis followed by bidirectional sequencing. Mutation screening of HRAS gene identified a novel "bona-fide" mutation outside the classical HRAS "hot-spot" region for CS. Thirty-three patients with CFCS were screened for mutations in MEK1 and MEK2 genes. Three MEK1 and two MEK2 mutations were detected in six patients. To investigate the phenotypic spectrum and molecular diversity of germ line mutations affecting BRAF, which encodes a serine/threonine kinase functioning as a RAS effector frequently mutated in CFCS, subjects with a diagnosis of NS (n. 270), LS (n. 6), and CFCS (n. 33), and no mutation in PTPN11, SOS1, KRAS, RAF1, MEK1, or MEK2, were screened for the entire coding sequence of the gene. Besides the expected high prevalence of mutations observed among CFCS patients (52%), a de novo heterozygous missense change was identified in one subject with LS (17%) and five individuals with NS (1.9%). Our findings provide evidence for a wide phenotypic diversity associated with mutations affecting BRAF, and occurrence of a clinical continuum associated with these molecular lesions.

Papers published within the project

Calcagni G, Digilio MC, Sarkozy A, Dallapiccola B, Marino B. Familial recurrence of congenital heart disease: an overview and review of the literature. *Eur J Pediatr* 2007;166(2):111-6.

De Luca A, Sarkozy A, Consoli F, De Zorzi A, Mingarelli R, Digilio MC, Marino B, Dallapiccola B. Exclusion of Cx43 gene mutation as a major cause of criss-cross heart anomaly in man. *Int J Cardiol* 2009;16. [E-pub ahead of print].

Dentici ML, Sarkozy A, Pantaleoni F, Carta C, Lepri F, Ferese R, Cordeddu V, Martinelli S, Briuglia S, Digilio MC, Zampino G, Tartaglia M, Dallapiccola B. Spectrum of MEK1 and MEK2 gene mutations in cardio-facio-cutaneous syndrome and genotype-phenotype correlations. *Eur J Hum Genet* 2009;17(6):733-40.

Digilio MC, Sarkozy A, Capolino R, Chiarini Testa MB, Esposito G, de Zorzi A, Cutrera R, Marino B, Dallapiccola B. Costello syndrome: clinical diagnosis in the first year of life. *Eur J Pediatr* 2008;167(6):621-8.

Limongelli G, Pacileo G, Digilio MC, Calabro' P, Di Salvo G, Rea A, Miele T, Frigiola A, Sarkozy A, Dallapiccola B, Marino B, Calabro' R. Severe, obstructive biventricular hypertrophy in a patient with Costello syndrome: Clinical impact and management. *Int J Cardiol* 2008;130(3):108-10.

Limongelli G, Pacileo G, Marino B, Digilio MC, Sarkozy A, Elliott P, Versacci P, Calabro P, De Zorzi A, Di Salvo G, Syrris P, Patton M, McKenna WJ, Dallapiccola B, Calabro R. Prevalence and clinical significance of cardiovascular abnormalities in patients with the LEOPARD syndrome. *Am J Cardiol* 2007;100(4):736-41.

Limongelli G, Sarkozy A, Pacileo G, Calabrò P, Digilio MC, Maddaloni V, Gagliardi G, Di Salvo G, Iacomino M, Marino B, Dallapiccola B, Calabrò R. Genotype-phenotype analysis and natural history of left ventricular hypertrophy in LEOPARD syndrome. *Am J Med Genet A* 2008;146A(5):620-8.

Mascheroni E, Digilio MC, Cortis E, Devito R, Sarkozy A, Capolino R, Dallapiccola B, Ugazio AG. Pigmented villonodular synovitis in a patient with Noonan syndrome and SOS1 gene mutation. *Am J Med Genet A* 2008;146A(22):2966-7.

Piacentini G, Digilio MC, Sarkozy A, Placidi S, Dallapiccola B, Marino B. Genetics of congenital heart diseases in syndromic and non-syndromic patients: new advances and clinical implications. *J Cardiovasc Med (Hagerstown)* 2007;8(1):7-11.

Sarkozy A, Carta C, Moretti S, Zampino G, Digilio MC, Pantaleoni F, Scioletti AP, Esposito G, Cordeddu V, Lepri F, Petrangeli V, Dentici ML, Mancini GM, Selicorni A, Rossi C, Mazzanti L, Marino B, Ferrero GB, Silengo MC, Memo L, Stanzial F, Faravelli F, Stuppia L, Puxeddu E, Gelb BD, Dallapiccola B, Tartaglia M. Germline BRAF mutations in Noonan, LEOPARD, and cardiofaciocutaneous syndromes: molecular diversity and associated phenotypic spectrum. *Hum Mutat* 2009;30(4):695-702.

Sarkozy A, Digilio MC, Dallapiccola B. Leopard syndrome. *Orphanet J Rare Dis* 2008;3:13.

Sarkozy A, Schirinzi A, Lepri F, Bottillo I, De Luca A, Pizzuti A, Tartaglia M, Digilio MC, Dallapiccola B. Clinical lumping and molecular splitting of LEOPARD and NF1/NF1-Noonan syndromes. *Am J Med Genet A* 2007;143A(9):1009-11.